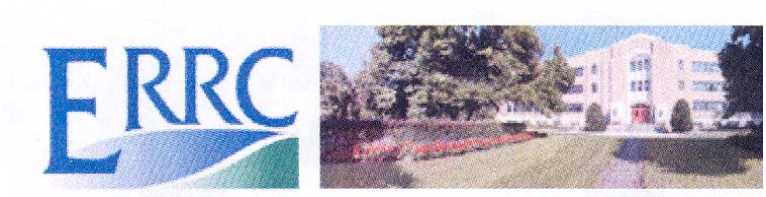

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Title: Irradiation D-Values for *Escherichia coli* O157:H7 and *Salmonella sp.* on Inoculated Broccoli Seeds and Effects of Irradiation on Broccoli Sprout Keeping Quality and Seed Viability

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Citation: Journal of Food Protection (2003) 66:(5) 760-766

Number: 7237

Irradiation *D*-Values for *Escherichia coli* O157:H7 and *Salmonella* sp. on Inoculated Broccoli Seeds and Effects of Irradiation on Broccoli Sprout Keeping Quality and Seed Viability†

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MS 02-131: Received 23 April 2002/Accepted 13 September 2002

ABSTRACT

Like alfalfa sprouts, broccoli sprouts can be a vehicle for bacterial pathogens, which can cause illness when they are consumed. The gamma irradiation process was used to reduce numbers of bacterial pathogens on broccoli sprouts and seeds, and the effect of this process on the seeds was studied. The irradiation destruct values for *Salmonella* sp. and for strains of *Escherichia coli* O157:H7 inoculated on broccoli seeds were determined. Results obtained in this study indicate that a dose of 2 kGy reduced total background counts for broccoli sprouts from 10^6 to 10^7 CFU/g to 10^4 to 10^5 CFU/g and increased the shelf life of the sprouts by 10 days. Yield ratio (wt/wt), germination percentage, sprout length, and thickness were measured to determine the effects of various irradiation doses on the broccoli seeds. Results show a decreased germination percentage at a dose level of 4 kGy, whereas the yield ratio (wt/wt), sprout length, and thickness decreased at the 2-kGy dose level. The radiation doses required to inactivate *Salmonella* sp. and strains of *E. coli* O157:H7 were higher than previously reported values. *D*-values, dose required for a 1-log reduction, for the nonvegetable and vegetable *Salmonella* sp. isolates were 0.74 and 1.10 kGy, respectively. The values for the nonvegetable and vegetable isolated strains of *Escherichia coli* O157:H7 were 1.43 and 1.11 kGy, respectively. With the irradiation process, a dose of up to 2 kGy can extend the shelf life of broccoli sprouts. A dose of >2 kGy would have an adverse effect on the broccoli seed and decrease the yield of broccoli sprouts.

In addition to the increased nutrient value of sprouted seeds *(7, 11, 15)*, other health benefits are associated with the consumption of certain sprout varieties, and the broccoli sprout is one variety that is said to offer such benefits *(5, 8)*. Sprouts of the *Brassica* genus contain isothiocyanates, which are potent inducers of enzymes that protect against chemical carcinogens *(8)*. The principal enzyme inducer in a 3-day-old broccoli sprout is either glucoraphanin or sulforaphane, which when consumed in small quantities may help protect against the risk of cancer *(8, 26)*. Nonleafy vegetables like broccoli have been reported to contain 1,000 to 2,000 μg of phylloquinone (vitamin K_1) per kg, which represents an added benefit *(5)*.

Like alfalfa sprouts, broccoli sprouts are generally consumed raw. Raw vegetables, including sprouts, can support the growth of microorganisms *(1, 21)*. Beuchat *(4)* listed the pathogenic microorganisms and the raw produce from which they have been isolated. *Escherichia coli* O157:H7 and *Salmonella*, associated with foodborne illnesses, have been isolated from raw sprouts *(16)*. The sprouting process, with its moist environment and available nutrients, is ideal for both sprout and microbial growth, including the growth of foodborne pathogens *(16)*. The fragile nature of the sprouts makes the use of any postharvest disinfectant step impractical. Thayer and Rajkowski *(24)* reviewed the application of ionizing irradiation of fresh produce to alter ripening rates, to control insect infestation, and to increase shelf life. Rajkowski and Thayer *(18)* reported that gamma radiation could be used to reduce both *Salmonella* sp. and strains of *E. coli* O157:H7 on sprouts. Their data showed that the radiation *D*-values, dose required for a 1-log reduction, for these pathogens were similar to values previously reported for meat products and that a 2-kGy dose was sufficient to obtain a 5-log reduction of both pathogens on sprouts. Rajkowski and Thayer *(19)* used a gamma irradiation process at a dose level of 2 kGy to increase the shelf life of alfalfa sprouts by 10 days.

Salmonella sp. and strains of *E. coli* O157:H7 were isolated from seeds used for sprouts *(16)*. According to the U.S. Food and Drug Administration (FDA), the consumption of pathogen-contaminated sprouts can be a potential health hazard *(16)*. In order to assure that sprouts are pathogen-free, the FDA recommends the use of a 20,000-ppm sodium hypochlorite wash for seeds prior to sprouting *(16)*. This wash is for the seed surface only and cannot guarantee the removal of pathogens in cracks or under the seed coat. In 2000, the irradiation process was approved for use on sprout seeds for doses of up to 8 kGy *(25)*. Rajkowski and Thayer *(19)* reported on the minimal effect of irradiation

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on the germination percentage for the irradiated alfalfa seed. With both laboratory and commercial methods for sprouting, the yield ratio (wt/wt) was acceptable up to the 2-kGy dose level *(19)*. There are no published data on the effect of irradiation on seed germination, yield ratios, or radiation *D*-values for broccoli seeds.

This study was conducted to determine whether the same increase in shelf life could be achieved for broccoli sprouts with an irradiation dose of 2 kGy. Broccoli seeds were also studied to determine the effects of irradiation on germination, yield, and growth. The radiation *D*-values for both nonproduce and produce pathogen isolates of *Salmonella* sp. and *E coli* O157:H7 strains inoculated on broccoli seeds were determined.

MATERIALS AND METHODS

Irradiation of samples. All samples (commercial packaged sprouts and seeds) were irradiated with a self-contained ^{137}Cs gamma radiation source with a dose rate of ca. 0.1 kGy/min. The dose rate was established with alanine transfer dosimeters from the National Institute of Standards and Technology, Gaithersburg, Md. Variation in the dose absorbed by the experimental samples was minimized by the placement of the samples within a uniform area of the radiation field. The actual dose was verified by dosimeter alanine pellet readings on an EPR analyzer (EMS 104 EPR, Bruker, Rheinstellen, Germany). The sample temperature was monitored continuously during irradiation and maintained through the injection of the gas phase of liquid nitrogen into the irradiation chamber.

Preparation of sprouts for keeping quality study. Within 24 h after harvesting and packaging, market packed broccoli sprouts (net weight 85.05 g) were purchased from a local grower. The sprouts were kept refrigerated (at 4 to 8°C) during transportation and storage. After the broccoli sprouts were irradiated with a dose of 2 kGy at 20°C, the controls and the irradiated sprouts were kept refrigerated (at 4 to 8°C) until they were used.

Microbiological analysis. On the day of irradiation and at weekly intervals, both a control and an irradiated market package of the broccoli sprouts were analyzed for total aerobic and coliform counts. After 1:10 dilution with buffered peptone water (Difco Laboratories, Detroit, Mich.) and serial dilution with buffered peptone water, the total aerobic and coliform counts were obtained with the use of aerobic and *E. coli*/coliform Petrifilms (3 M Microbiology Products, St. Paul, Minn.), respectively. The films were incubated for 24 h at 37 ± 1°C before being hand counted according to the recommended procedure.

Visual keeping quality. Changes in appearance and microbial counts were followed as a function of the 2-kGy radiation treatment and storage time. The visual changes were recorded by photographing the sprouts after taking the microbial sample from the same market package.

Irradiation's effect on the germination, yield ratio, and growth of broccoli seeds. Broccoli seeds (*Brassica oleracea* var. *botrytis*) were obtained from Caudill Seed Co., Inc., Louisville, Ky. The broccoli seeds were irradiated at 1, 2, 3, 4, and 5 kGy. The germination percentage was determined for 100 seeds that were germinated according to the method described by the Association of Official Seed Analysts *(2, 3)*. The germination percentage was calculated for duplicate samples, and the calculation was repeated. The yield ratio was determined by sprouting a known amount of seeds with the EasyGreen Automatic Sprouter System (Seed and Grain Technology, Albuquerque, N.M.) and weighing the resulting sprouts after the prescribed growth time *(2, 3)*. The determination of the yield ratio (wt/wt) was repeated twice. At the end of each yield ratio determination, 100 sprouts were measured for hypocotyl thickness and sprout length with a Digimatic caliper (Mitutoyo Corp., Kanagawa, Japan).

Cultures. Nine nonvegetable isolates were used in this study. *Salmonella enterica* Dublin 15480, *S. enterica* Enteritidis 13076, *S. enterica* Newport 6962, *S. enterica* Senftenberg ATCC 8400, *S. enterica* Typhimurium 14028, and *Escherichia coli* O157:H7 strains 35150, 43889, and 43894 were obtained from the American Type Culture Collection, Rockville, Md. *E. coli* O157:H7 strain 93-437 was obtained from the Oregon Public Health Laboratory, Portland, Oreg., and *E. coli* O157:H7 strain ENT C9490 was obtained from the Centers for Disease Control and Prevention, Atlanta, Ga. The seven vegetable-related isolates (*S. enterica* Anatum F4317, *S. enterica* Stanley H0558, *S. enterica* Newport H1275, *S. enterica* Infantis F4319, and *E. coli* O157:H7 strains F4546, SEA13B88, and C7927) were obtained from Dr. William Fett, U.S. Department of Agriculture, Agricultural Research Service, Wyndmoor, Pa.

The identity and purity of each isolate were verified with Gram stains and reactions on the GNI card of the Vitek AMS Automicrobic System (bioMérieux Vitek, Inc., Hazelwood, Mo.). All cultures were maintained at 4°C on tryptic soy agar (TSA; Difco) slants. Working cultures were prepared with tryptic soy broth (TSB; Difco) and kept at 4°C. The day before seed inoculation, each isolate was cultured separately in 100 ml of TSB in a 500-ml baffled flask with agitation at 150 rpm on a rotary shaker for 18 h at 37 ± 1°C. The cultures were combined in equal volumes to make an inoculum cocktail. The cell concentrations for both nonvegetable and vegetable isolates were 10^6 to 10^7 CFU/ml. The cocktail volume used was 1 ml/g.

Seed inoculation. The broccoli seeds were packaged in polyester-lined filter stomacher bags (Seward Model 400, Lab Source, Chicago, Ill.), and the filter liner and bag were heat sealed before being sterilized with 25 kGy of irradiation at 20°C to remove microorganisms. Before inoculation, the polyester liner containing the seeds was removed from the outer bag. The seeds contained in the polyester filter were inoculated with the inoculum cocktail (1 ml/g of seed) for 1 min. Immediately after its removal from the inoculating solution, the outside of the bag containing the seeds was patted dry and then placed in a desiccator and vacuum sealed. The seeds remained in the vacuum desiccator until they were dry (about 2 days). Only after drying were the seeds removed from the polyester liner.

Radiation *D*-values for *E. coli* O157:H7 and *Salmonella* on broccoli seeds. Five grams of the inoculated seeds was weighed into filter-lined stomacher bags and heat sealed before irradiation was carried out. The inoculated seed samples received absorbed radiation doses of 0, 0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, and 2.8 kGy at 19 ± 1°C. Each study was repeated.

Microbiological analysis for determination of radiation *D*-values. A nonirradiated inoculated seed sample, along with the irradiated samples used for the determination of radiation *D*-values, was diluted with buffered peptone water (Difco) and stomached for 1 min with a Stomacher 400 (Tekmar Co., Cincinnati, Ohio). After serial dilution with 0.1% peptone water (Difco), the samples were plated on TSA with an automatic spiral plater (Autoplate 4000, Spiral Biotech, Inc., Bethesda, Md.). The plates were

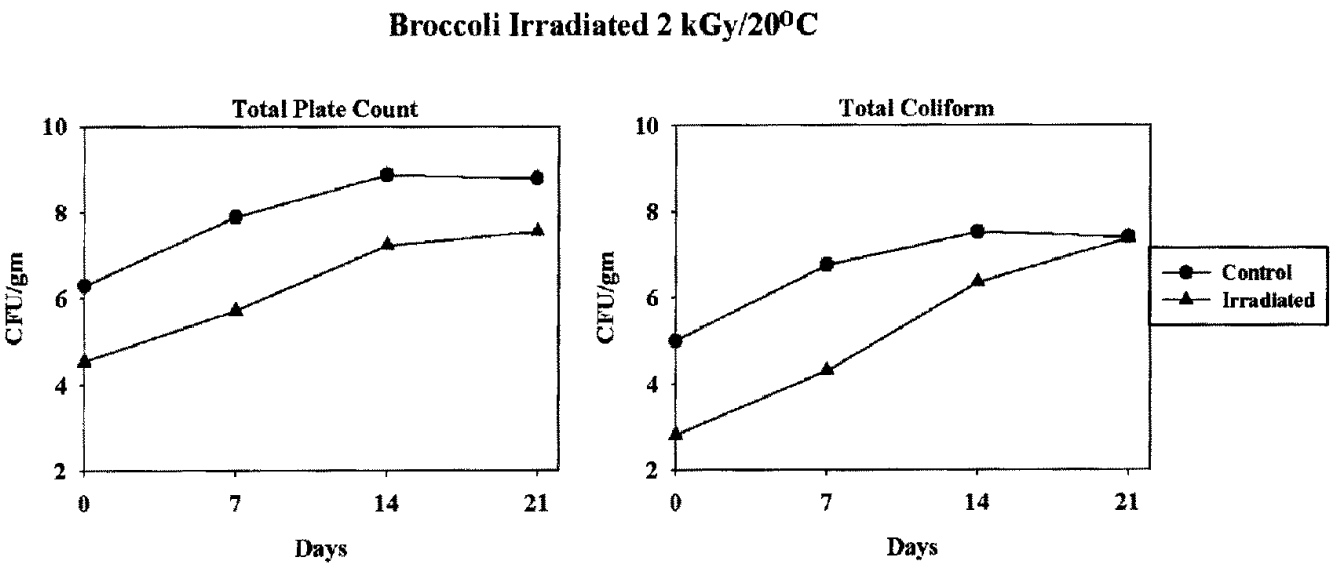

FIGURE 1. *Regrowth of total aerobic bacteria and coliforms on broccoli sprouts irradiated at 2 kGy.*

incubated for 24 h at 35 ± 1°C before the count was determined with a spiral laser colony scanner (Model 500A, Spiral Biotech).

Statistical analysis. For analysis, the average number of surviving CFU per gram was divided by the average zero-dose value (N_0) to obtain a survivor value (N/N_0). For subsequent calculations, the log survivor values ($\log[N/N_0]$) were used. To avoid possible shoulder effects, the N_0 values were not used, and a minimum of five values in the linear portion of the inactivation curves were used to calculate each regression *(20)*. The results of the two independent replicate studies were pooled, and the slope of the inactivation curve was determined by least-squares analysis. The radiation *D*-value was calculated from the slope. The general linear model procedure of the SAS statistical package *(20)* was used to perform statistical calculations. The regressions were tested for significant differences by analysis of covariance.

RESULTS AND DISCUSSION

Broccoli sprout keeping quality. The shelf life of fresh clamshell-packaged broccoli sprouts obtained from local sprout growers was increased by 10 days with irradiation at 2 kGy. A 10-day increase in shelf life has also been reported for irradiated alfalfa sprouts *(19)*. The aerobic background counts for alfalfa sprouts can vary from 10^5 to 10^8 CFU/g depending on the producer, the handling and harvesting practices used, and the climate during sprouting and transportation *(19)*. In this study, the range of total aerobic background counts on the fresh broccoli sprouts were between 10^6 and 10^7 CFU/g before irradiation at 2 kGy and between 10^4 and 10^5 CFU/g after irradiation. The total coliform counts for the broccoli sprouts before irradiation ranged from 10^5 to 10^6 CFU/g, and the counts and after irradiation ranged from 10^3 to 10^4 CFU/g. Regardless of when or where the sprouts were purchased, there was an average 2- to 3-log decrease in total aerobic counts and coliform counts, which is less extensive than the decrease reported for alfalfa sprouts *(19)*.

The representative curves for the total aerobic and coliform counts presented in Figure 1 show the count increases during storage after irradiation. These curves are similar to those for the count increases for the alfalfa sprouts irradiated at 2 kGy *(19)*. Increases in total plate counts for irradiated and control samples occurred with storage at 4°C, but the count for the irradiated sample never increased to the level of that of the control, whereas the total coliform counts for the irradiated sample were below those for the control sample for the first 2 weeks of storage and these counts were the same for both samples after 21 days. The growth of coliform bacteria in the irradiated samples may be due to the reduction in lactic acid bacteria. Garcia-Gimeno and Zurera-Cosano *(12)* reported that the lactic acid bacteria level could be related to spoilage and shelf life when the lactic acid bacterium level reached 10^6 CFU/g. In this study, the lactic acid bacterium levels were not determined, and spoilage of the nonirradiated samples did occur when the total aerobic counts were $\geq 10^8$ CFU/g.

Samples of the broccoli sprouts were photographed at each sampling time to document the deterioration of the unirradiated and the irradiated products during storage. The photographs (Fig. 2) of the samples at 0 and 21 days of storage show the amount of deterioration of the unirradiated broccoli sprouts. After 3 weeks of storage, the broccoli sprouts curled and were difficult to separate and exhibited obvious browning and mycelium growth. These results are similar to those reported for alfalfa sprouts *(19)*, indicating that the shelf life of the broccoli sprouts irradiated at 2 kGy was extended; after 3 weeks, these sprouts were just beginning to show signs of deterioration.

Effect of irradiation dose on broccoli seeds. The effects of various irradiation doses from 1 to 5 kGy were studied to determine their effects on germination percentage and yield ratio (wt/wt) for broccoli seeds and on sprout length and hypocotyl thickness after 4 days of sprout growth. The germination percentage was >90% for dose levels of ≤3 kGy (Table 1). A comparison of the germination percentages for the broccoli seeds and those for the alfalfa seeds at irradiation doses of up to 5 kGy reveals that

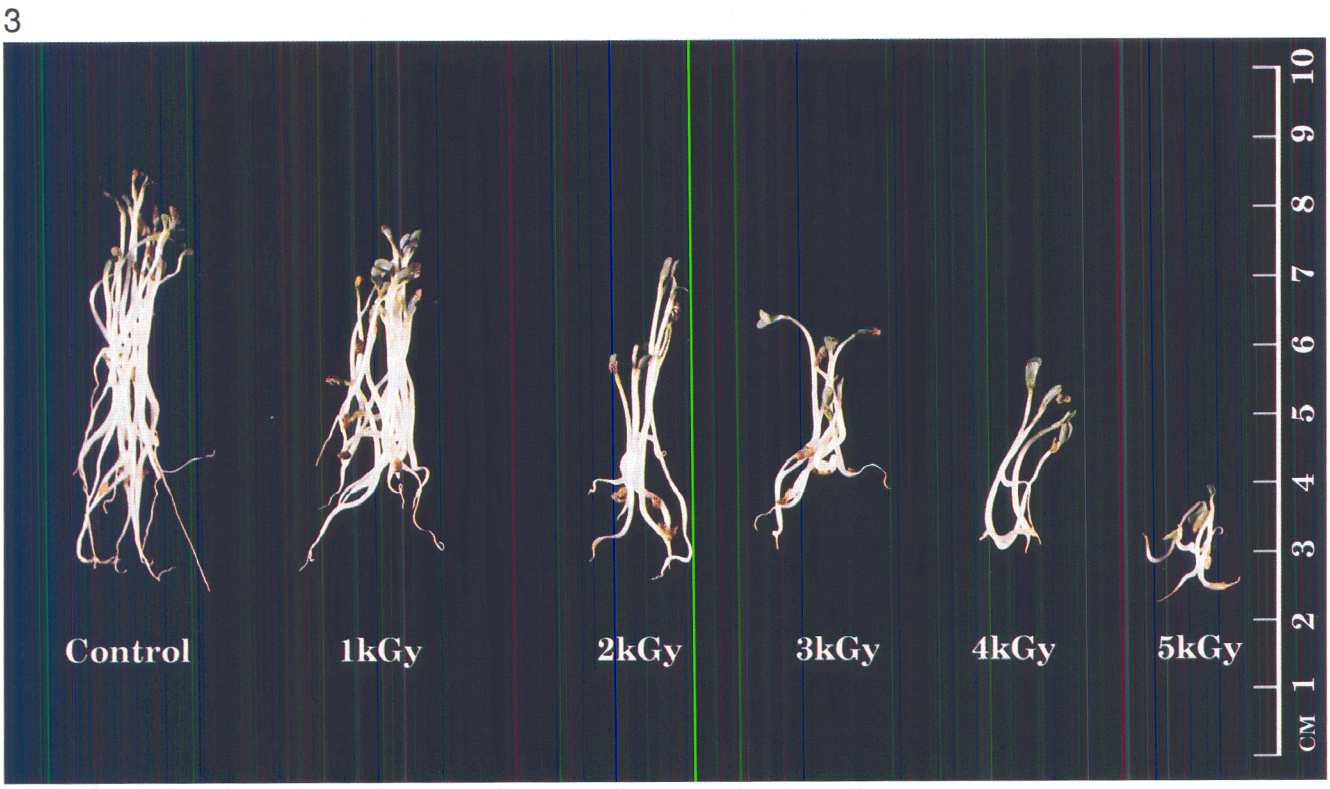

FIGURE 2. *Keeping quality of irradiated broccoli sprouts compared with that of unirradiated sprouts at 0 and 21 days of storage at 4°C.*

FIGURE 3. *Effect of irradiation of seeds on yield of broccoli sprouts after 4 days of growth.*

broccoli seeds were more affected by irradiation at the lower dose of 3 kGy, whereas alfalfa seeds treated with a dose of 5 kGy exhibited 98% germination *(19)*. The decreases in the length and thickness of broccoli sprouts (Table 1) also reflect the adverse effect of irradiation on the seeds as the dose increased. Other researchers have reported on this decrease in sprout length (yield) for irradiated lentil, wheat, rice, peanut, maize, soybean, red bean, mung bean, and cowpea seeds *(6, 13, 17, 27)*. These researchers propose that the germination test can be used to determine whether seeds have been irradiated on the basis of the visual decrease in growth relative to that of the control.

There was a decrease in the yield ratio (wt/wt) as the irradiation dose increased (Table 1), and this decrease corresponded to the decrease in the germination percentage. Figure 3 illustrates the decrease in broccoli sprout size (less growth), which also resulted in lower yield ratios as the dose rate increased. The yield ratio of ≥10:1 (wt/wt) required by the sprout producers was achieved only with a ≤2-kGy level after 6 days of growth.

TABLE 1. *Germination percentages, yield ratios, sprout lengths, and thicknesses after 4 days of growth for irradiated broccoli seeds*

Irradiation doses (kGy)	% germination	Yield ratio (wt/wt)	Sprout length (mm)[a]	Sprout thickness (mm)[a]
0	97	12.3	30 ± 5.7	0.73 ± 0.11
1	95	11.1	14 ± 1.4	0.64 ± 0.17
2	91	9.1	11 ± 1.4	0.64 ± 0.11
3	91	8.7[b]	14 ± 2.8	0.63 ± 0.04
4	88	7.2	7 ± 1.4	0.45 ± 0.06

[a] Average ± standard deviation for 100 sprouts.
[b] Yield ratio unacceptable for commercial sprouter.

Radiation *D*-value for *E. coli* O157:H7 and *Salmonella* sp. inoculated on broccoli seeds. Radiation *D*-values were obtained from the inactivation curves for the vegetable isolates of *E. coli* O157:H7 and *Salmonella* on broccoli seeds and are presented in Figures 4 and 5, respectively. The radiation *D*-value for the nonvegetable isolates of *E. coli* O157:H7 was 1.43 ± 0.07 kGy, which is statistically significantly higher ($P > 0.05$) than that determined for the vegetable isolates (1.11 ± 0.12 kGy) (Table 2). Higher *D*-values for *E. coli* O157:H7 meat isolates than for vegetable isolates was previously observed *(18)*.

The radiation *D*-values for the nonvegetable and vegetable *Salmonella* sp. isolates were 0.74 ± 0.04 and 1.11 ± 0.04 kGy, respectively (Table 2). For the vegetable *Salmonella* sp. isolates, *D*-values were statistically higher ($P > 0.05$). This difference was not observed in the study involving radish sprouts. In that study, the *D*-values for the nonvegetable isolates were statistically significantly ($P > 0.05$) higher (Table 2) *(18)*.

The broccoli seed radiation *D*-values for the nonvegetable *E. coli* O157:H7 and *Salmonella* sp. isolates were higher than the *D*-values reported for the same isolates inoculated on radish sprouts *(18)* and meat products *(22, 23)*. This increase in radiation *D*-values is probably due to the low water content of the dry seeds relative to that of the moist sprout or meat products and to antioxidants located on the seed coat. The results of the present study indicate that the irradiation of broccoli seeds at >2 kGy would adversely affect the seeds' viability and would result in only a 90% reduction of *E. coli* O157:H7 and *Salmonella* on the seeds.

CONCLUSIONS

The use of irradiation at up to 8 kGy to control bacterial pathogens on seeds for sprouting has been approved *(25)*. In this study, it was found that as the dose increased to 3 kGy, the germination percentage for broccoli seeds was not affected. The yield ratio (wt/wt) was reduced at a dose of 2 kGy, and extending the growth time by a day did not improve the yield ratio for seeds irradiated at ≥3 kGy, which may not be acceptable to a sprout producer. The 5-log kill required by the FDA cannot be achieved by irradiation alone. At a dose of 3 kGy, only a 2.7-log kill for the pathogen groups studied was observed for the dried inoculated broccoli seeds. Studies are needed to determine if the antioxidants that constitute a health benefit of eating broccoli sprouts protect the bacteria on the seed, as indicated by the higher radiation *D*-values observed in the present study. Studies are needed to determine whether a combination of a liquid disinfectant (or another agent) and irradiation provides a lower-risk broccoli sprout if the seeds are contaminated with pathogens.

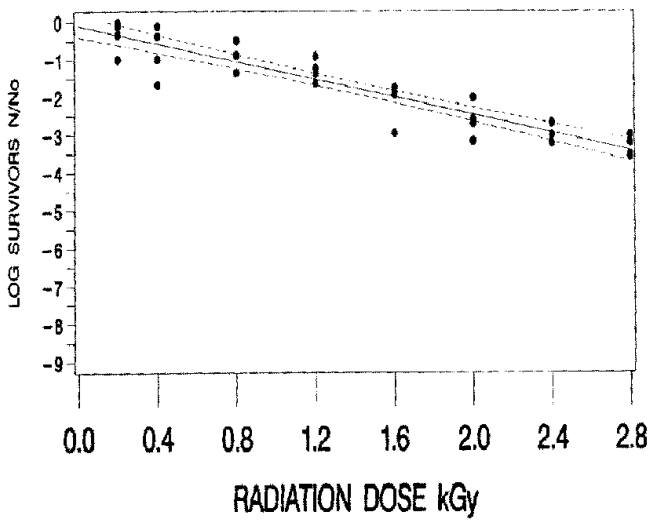

FIGURE 4. *Survival of a mixture of* E. coli *O157:H7 strains F4546, SEA13B88, and C7927 on inoculated raw broccoli seeds after gamma irradiation.*

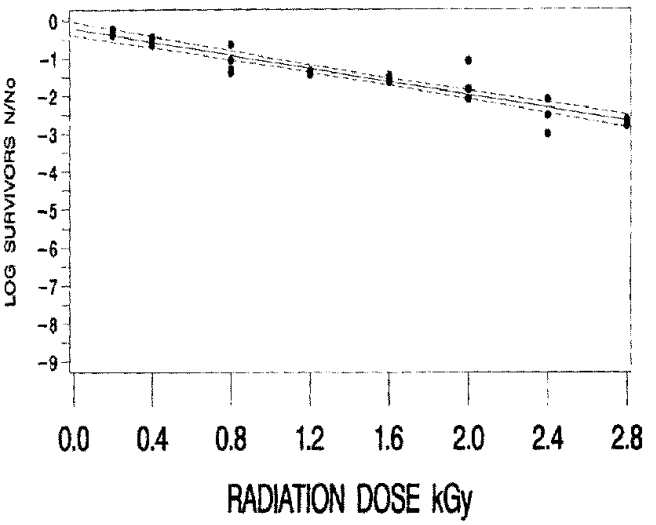

FIGURE 5. *Survival of a mixture of* Salmonella *Anatum,* Salmonella *Stanley,* Salmonella *Newport, and* Salmonella *Infantis on inoculated raw broccoli seeds after gamma irradiation.*

TABLE 2. *Comparison of radiation* D*-values for* E. coli *O157:H7 and* Salmonella *sp. on broccoli seeds, radish sprouts, and meat products*

Microorganism	Isolate type	Radiation *D*-value (kGy; mean ± SD) for[a]: Broccoli seeds	Radish sprouts[b]	Meat product[c]
E. coli O157:H7	Meat[d]	1.43 ± 0.07 B	0.34 ± 0.01 A	0.30 ± 0.02 A
	Vegetable[e]	1.11 ± 0.12 C	0.30 ± 0.02 A	
Salmonella	Meat[f]	0.74 ± 0.04 D	0.54 ± 0.02 A	0.53 ± 0.02 A
	Vegetable[g]	1.11 ± 0.04 C	0.40 ± 0.02 A	

[a] Values with the same letter are not significantly different ($P = 0.05$).
[b] Results taken from National Advisory Committee on Microbiological Criteria for Foods *(16).*
[c] Results taken from Rajkowski and Thayer *(19, 20).*
[d] *E. coli* O157:H7 strains ATCC 35150, ATCC 43889, ATCC 43894, and ENT C9490.
[e] *E. coli* O157:H7 strains F4546, SEA13B88, and C7927.
[f] *S. enterica* Dublin ATCC 15480, *S. enterica* Enteritidis ATCC 13076, *S. enterica* Newport ATCC 6962, *S. enterica* Senftenberg ATCC 8400 and *S. enterica* Typhimurium ATCC 14028.
[g] *S. enterica* Anatum F4317, *S. enterica* Stanley H0558, *S. enterica* Newport H1275, and *S. enterica* Infantis F4319.

Rajkowski and Thayer *(19)* reported that 4- to 5-log reductions of both *Salmonella* sp. and *E. coli* O157:H7 were obtained when sprouts were irradiated at 2 kGy. In this study, we found that irradiation at 2 kGy, the dose required for a 5-log reduction of the bacterial pathogens on sprouts, extended the shelf life of broccoli sprouts by 10 days. Farkas et al. *(10)* showed that the keeping quality of cut pepper and carrots was increased by a radiation dose of 1 kGy and that after 10 days of storage, β-carotene levels were higher in the irradiated samples. Fan and Thayer *(9)* also reported that β-carotene levels were higher in irradiated alfalfa sprouts and that irradiation did not consistently affect chlorophyll content or color. Further work is needed to determine the effect of irradiation on the quality of broccoli sprouts. Modified atmosphere packaging is another method that has been developed to control the microbiological storage stability of fresh-cut produce *(14).* Studies are needed to determine whether the effect of a combination of irradiation and modified atmosphere packaging could increase the shelf life of broccoli sprouts while assuring safe, pathogen-free sprouts for the consumer.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the cooperation of Gail Pippin of Windy Hollow Farms for providing the fresh broccoli sprouts used in this study, Bob Rust and Dan Caudill for their contributions, and Dr. John Philips for statistical aid. Gina Bates, Dana Drazul, Robert Richardson, and Kimbrilee Snipes did the laboratory technical work.

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